

STATUS OF MULTI DRUG RESISTANT *STAPHYLOCCUS AUREUS* IN BUFFALOES OF EASTERN PLAIN ZONE OF UTTAR PRADESH HAVING SUBCLINICAL MASTITIS

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ABSTRACT

The present study was conducted to study the antibiogram pattern with special reference to multi drug resistant *S. aureus* in buffaloes of eastern plain zone of Uttar Pradesh, India. A total of 200 milk samples were collected from the buffaloes of Sultanpur and Faizabad district of U.P. and processed for isolation of *Staphylococcus aureus*. *S. aureus* was successfully isolated from 140 milk samples using Mannitol Salt Agar. Out of 140 isolates none of the *S. aureus* isolates from mastitic milk were 100% sensitive to any antibiotic. Multi Drug Resistance was observed in 100% buffaloes of eastern plain zone for atleast 2 drugs. Highest 133 isolates (95%) were resistant to Cefotaxime, 120 isolates (85.72%) were resistant to Ampicillin, 113 isolates (80.72%) were resistant to Ceftazidime-Tazobactam, 27 isolates (18.58%) showed resistance against Gentamicin and Methicillin, 33 isolates (23.58%) showed resistance against Cloxacillin followed by Tetracycline where 20 isolates (14.28%) showed only resistance. Least resistance (33 isolates,

9.29%) was exhibited for cloxacillin, that proved to be the most effective antibiotic. Among the 140 *S. aureus* isolated from mastitic milk, 27 isolates (19.28%) exhibited resistance against 2 antibiotics, 20 (14.28%) isolates were resistance to 3 antibiotics, 33 isolates (23.58%) were resistant to 4 antibiotics. 40 (28.57%) isolates were resistant to 5 antibiotics. Seven (5%) isolates each were resistant to 6 and 7 antibiotics. 6 isolates (4.28%) were resistant to maximum 8 antibiotics On antibiogram mapping it was found that, the most effective antibiotic against *S. aureus* in this region in subclinical mastitis was Ofloxacin followed by Tetracycline, Methicillin and Gentamicin.

Keywords: *Bubalus bubalis*, buffaloes, mastitis, multi drug resistance

INTRODUCTION

Subclinical form of mastitis, a hidden danger to dairy industry with higher rate of prevalence, is economically destructive and

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clinically elusive disease due to its camouflaged clinical manifestations. Although mastitis is a multi etiological complex disease, but involvement of bacteria, particularly *Staphylococci*, comprising coagulase-positive *Staphylococci* (CPS) and coagulase-negative *Staphylococci* (CNS) needs special mention. Staphylococci are the predominant pathogens, which show wide variation in the rate of prevalence in different populations and their non-response and/or differential response of the pathogens to the available antibiotics (Harini *et al.*, 2011; Tyagi *et al.*, 2013).

A good amount of literature is available on the antibiogram of different mastitogens and workers from India have also reported *Staphylococci* and *Streptococci* to be the main etiological agents of mastitis in different parts of the country (Chavan *et al.*, 2007; Sharma and Sindhu, 2007; Sindhu *et al.*, 2010). Studies conducted by several workers (Chavan *et al.*, 2007; Roychoudhury and Dutta, 2009; Sharma *et al.*, 2010) have showed increased resistance towards different traditional and newly introduced antibiotics. The present investigation was planned to study to find out the drug resistance pattern of *S. aureus* in the buffaloes of eastern plain zone of Uttar Pradesh.

MATERIALS AND METHODS

Clinical inspection of the udder

Udders of the buffaloes were examined by visual inspection and palpation for the presence of any lesion, pain, heat and swelling. In addition, milk from each quarter was withdrawn and checked for any change in colour and consistency.

Collection of milk sample

Milk samples were collected from

Sultanpur and Faizabad districts of Eastern Plain Zone. From each districts 2 blocks were chosen. From each block two villages were chosen. 25 milk samples were collected from each village. In this way a total of 200 milk samples were collected. The affected quarter was washed with tap water, dried properly and the teat and was swapped with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was then collected aseptically from a mastitic quarter into sterile universal bottle after discarding the first 3 to 4 milking streams. Sample from each quarter were transported on ice packs to the bacteriology laboratory and were immediately cultured and stored at 4°C for a maximum of 24 h until cultured on standard bacteriological media.

The milk sample were screened for mastitis using California mastitis test and the samples showing strong positive reaction were selected for isolation of *Staphylococcus aureus*.

Bacteriological examination of samples

The milk samples were subjected to bacteriological examination as per the method prescribed by National committee for clinical laboratory standards (NCCLS,1997). In the current study antibiotic resistance pattern of 140 isolates *S. aureus* was tested against 9 antibiotics *viz.* Ampicillin, Cloxacillin, Tetracycline, Oxacillin, Cefotaxime, Ofloxacin, Gentamicin, Ceftazidime-Tazobactam and Methicillin.

RESULTS AND DISCUSSION

On testing the antibiotic susceptibility it was found that there is 100% multiple drug resistance (MDR) in buffaloes of eastern plain zone of Uttar Pradesh. Antibiogram mapping revealed that the most effective antibiotic against

S. aureus in this region against subclinical mastitis is Ofloxacin followed by Tetracycline. Methicillin and Gentamicin were susceptible to 113 isolates (80.72%).

Out of 140 isolates none of the *S. aureus* isolates from mastitic milk were 100% sensitive to any antibiotic (Figure 1, Table 1). Highest 133 isolates (95%) exhibited resistance against Cefotaxime which was highest among 9 antibiotics used followed by Ampicillin where 120 isolates (85.72%) were found resistant. 113 isolates (80.72%) were resistant to Ceftazidime Tazobactam, 100 isolates (71.43%) were resistant to Oxacillin. Twenty seven isolates (18.58%) showed resistance against Gentamicin. Thirty two isolates (22.86%) showed resistance against Cloxacillin followed by Tetracycline where only 20 isolates (14.28%) showed resistance. Least resistance (33 isolates, 9.29%) was exhibited for cloxacillin, that proved to be the most effective antibiotic.

Among the 140 *S. aureus* isolated from mastitic milk, 27 isolates (19.28%) exhibited resistance against 2 antibiotics, 20 (14.28%) isolates were resistance to 3 antibiotics, 33 isolates (23.58%) were resistant to 4 antibiotics. 40 (28.57%) isolates were resistant to 5 antibiotics. 7 (5%) isolates each were resistant to 6 and 7 antibiotics. 6 isolates (4.28%) were resistant to maximum 8 antibiotics (Figure 2, Table 2).

The non-judicious use of antibiotics like ampicillin, oxacillin and methicillin contribute to the increasing occurrence of antibiotic resistant strains in bovines with mastitis. These strains in intramammary dissemination often produce incurable severe intra herd infections (Moon *et al.*, 2007). Resistance of *S. aureus* to antimicrobial agents can complicate treatment of its infections (Lowy, 2003). At present, there is scarcity of reports about occurrence of multi drug resistance

(MDR) isolates from this part of India. Multiple drug resistance has emerged among bacterial infection in last few years. There are large numbers of cases from different parts of the world that describe increased trend of developing multiple resistance strains (Shana *et al.*, 2009; Rinsky *et al.*, 2013). According to Ankita (2015), 7 isolates were resistance to 2 antibiotics 3 isolates were resistant to 3 antibiotics, while 1 isolate each showed resistance to 4 and 5 antibiotics. Tyagi *et al.* (2011) also reported 23.52% isolates to be resistance to 2 antibiotics, 19.11% isolates to be resistance to 3 antibiotics, 2 isolates to be resistance to 4 antibiotics, 1 isolates to be resistance to 5 antibiotics among 36 isolates.

The multiple drug resistance in Staphylococcal was also reported by other workers (Ombui *et al.*, 2000; Gentilini *et al.*, 2002; Rajala-Schultz *et al.*, 2004; Adwan *et al.*, 2006; Wang *et al.*, 2013; Kumar *et al.*, 2010).

WHO report 2002 suggested that overuse and misuse of antibacterial agents could be responsible as a major selective force leading to the development of bacterial resistance. In the recent past, it was very strongly suggested that the indiscriminate use of antibiotic must be avoided and for clinical treatment, a proper analysis of antibiotic profile of the bacteria should be ascertained before using any antibiotic (Thapa *et al.*, 2007; Sumathi *et al.*, 2008; Aire-de-sousa *et al.*, 2007; Sudhakar *et al.*, 2009; Kumar *et al.*, 2010).

REFERENCES

- Aires-de-Sousa, M., C.E. Parente, O. Vieira-da-Motta, I.C. Bonna, D.A. Silva and H. De Lencastre. 2007. Characterisation of *Staphylococcus aureus* isolates from

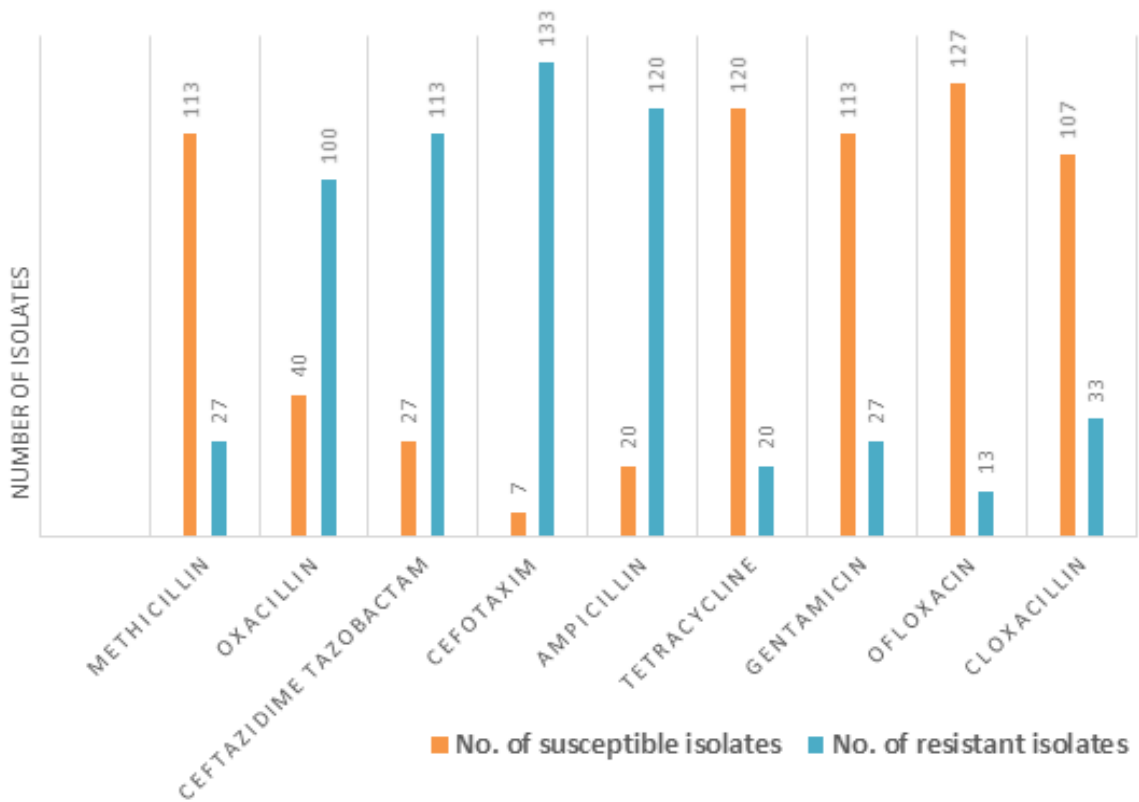


Figure 1. Graph showing number of sensitive and resistance isolates against antibiotics used.

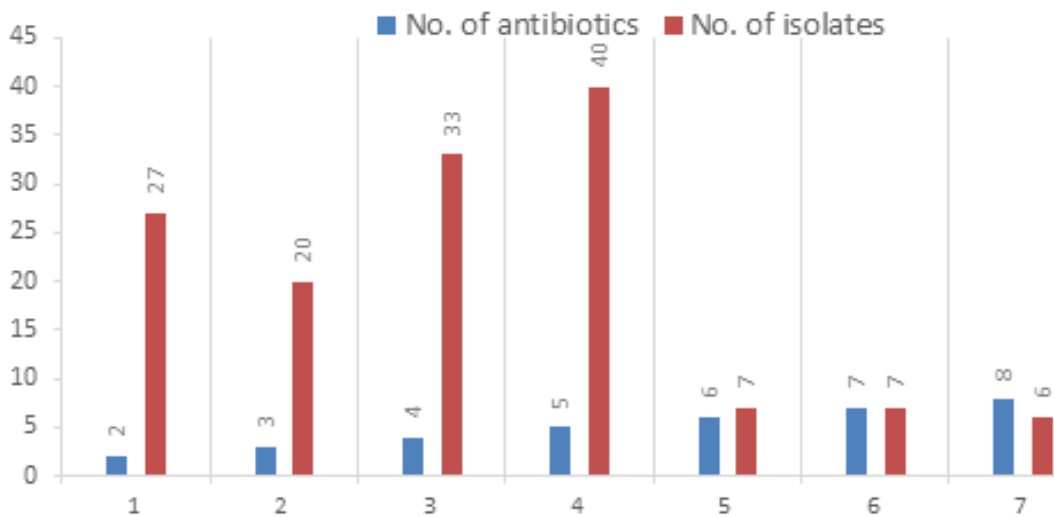


Figure 2. Graph showing multi drug resistance pattern.

Table 1. Sensitivity range of *S. aureus* isolates from buffalo milk.

S. No.	Antibiotic used	Code of antibiotic disc	Concentration per disc (mcg)	<i>S. aureus</i>	
				No. of susceptible isolates (%)	No. of resistant isolates (%)
1	Methicillin	MET	4	113 (80.72%)	27 (19.28%)
2	Oxacillin	OX	1	40 (28.57%)	100 (71.43%)
3	Ceftazidime/Tazobactam	CAT	80/10	27 (19.28%)	113 (80.72%)
4	Cefotaxim	CTX	10	7 (5%)	133 (95%)
5	Ampicillin	AMP	2	20 (14.28%)	120 (85.72%)
6	Tetracycline	TE	30	120 (85.72%)	20 (14.28%)
7	Gentamicin	GEN	10	113 (80.72%)	27 (19.28%)
8	Ofloxacin	OF	5	127 (90.71%)	13 (9.29%)
9	Cloxacillin	COX	10	107 (76.42%)	33 (23.58%)

Table 2. Multiple drug resistance pattern of *S. aureus* from mastitic milk.

Organism	No. of isolate tested	Combination of resistance antibiotic		Name of antibiotic (Number)
		No. of antibiotics	No. of isolates	
<i>S. aureus</i>	140	2	27 (19.28%)	OX + CTX (7) CAT + CTX (7) AMP + CTX (13)
		3	20 (14.29%)	OX + CAT + CTX (7) AMP + CAT + CTX (13)
		4	33 (23.58%)	OX + CAT + CTX + AMP (26) OX + AMP + TE + MET (7)
		5	40 (28.57%)	OX + CAT + CTX + AMP + GEN (20) OX + CAT + CTX + AMP + MET (7) OX + CAT + CTX + AMP + COX (13)
		6	7 (5%)	CAT + CTX + AMP + TE + COX + MET (7)
		7	7 (5%)	OX + CAT + CTX + AMP + TE + OF + COX (7)
		8	6 (4.28%)	OX + CAT + CTX + AMP + COX + OF + GEN + MET (6)

- buffalo, bovine, ovine and caprine milk samples collected in Rio de Janeiro State, Brazil. *Appl. Environ. Microbiol.*, **73**(12): 3845-3849. DOI: 10.1128/AEM.00019-07
- Ankita, Y. 2015. *Characterization of Staphylococcus aureus associated with bovine mastitis in reference to Methicillin resistance and antibiogram*. M.V.Sc. Thesis, N.D. University of Agriculture and Technology, Kumarganj, Faizabad (UP), India.
- Chavan, V.V., S.U. Digraskar, S.N. Dhonde and P.B. Hase. 2007. Observation on bubaline subclinical mastitis in and around Parbhani. *Indian J. Field Vet.*, **3**: 50.
- Coelho, S.M.O., E. Reinoso, I.A. Pereira, L.C. Soares, M. Demo, C. Bogin and M.M. Souza. 2009. Viulence factors and antimicrobial resistance of *S. aureus* isolated from bovine mastitis in Rio De Janeiro. *Pesqui. Vet. Brasil.*, **29**(5): 369-374. DOI: 10.1590/S0100-736X2009000500002
- Gentilini, E., G. Denamiel and A. Betancor. 2002. Antimicrobial susceptibility of coagulase-negative *Staphylococci* isolated from bovine mastitis in Argentina. *J. Dairy Sci.*, **85**(8): 1913-1917. DOI: 10.3168/jds.S0022-0302(02)74267-7
- Harini, H. and B.R. Sumathi. 2011. Screening of bovine milk samples for sub-clinical mastitis and antibiogram of bacterial isolates. *Vet. World.*, **4**(8): 358-359. DOI: 10.5455/vetworld.2011.358-359
- Kumar, A., A. Rahal, S.K. Dwivedi and M.K. Gupta. 2010. Bacterial prevalence and antibiotic resistance profile from bovine mastitis in Mathura, India. *Egyptian Journal of Dairy Science*, **38**(1): 31-34. Available on: <https://www.cabdirect.org/cabdirect/FullTextPDF/2010/20103207687.pdf>
- Lowy, F.D. 2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. *J. Clin. Invest.*, **111**(9): 1265-1273. DOI: 10.1172/JCI18535
- Moon, J.S., A.R. Lee, H.M. Kang, E.S. Lee, Y.S. Joo, Y.H. Park, M.N. Kim and H.C. Koo. 2007. Antibiogram and coagulase diversity in Staphylococcal enterotoxin-producing *Staphylococcus aureus* from bovine mastitis. *J. Dairy Sci.*, **90**(4): 1716-1724. DOI: 10.3168/jds.2006-512
- NCCLS. 2004. *Performance Standards for Antimicrobial Disk Susceptibility Testing; Fourteenth Informational Supplement*. NCCLS Document M100-514, Wayne, Pennsylvania, USA.
- Ombui, J.N., A.M. Kimotho and J.G. Nduhiu. 2000. Antimicrobial resistance patterns and plasmid profiles of *S. aureus* isolated from milk and meat. *East Afr. Med. J.*, **77**(9): 463-467. DOI: 10.4314/eamj.v77i9.46688.
- Rajal-Schulyz, P.J., K.L. Smith, J.S. Hogan and B.C. Love. 2004. Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. *Vet. Microbiol.*, **102**(1-2): 33-42. DOI: 10.1016/j.vetmic.2004.04.010
- Rinsky, J.L., M. Nadimpalli, S. Wing, D. Hall, D. Baron, L.B. Price, J. Larsen, M. Stegger, J. Stewart and C.D. Heaney. 2013. Livestock associated methicillin and multidrug resistant *Staphylococcus aureus* is present among industrial, not antibiotic-free livestock operation workers in North Caroline. *Plos One.*, **8**(7): e67641. DOI: 10.1371/journal.pone.0067641
- Roychoudhury, P. and T.K. Dutta. 2009. Prevalence and antibiotic sensitivity pattern of bacteria

- from bovine mastitis in Mizoram. *Indian J. Anim. Sci.*, **79**(5): 483-484.
- Sharma, A. and N. Sindhu. 2007. Occurrence of clinical and sub clinical mastitis in cows and buffaloes in State of Haryana (India). *Ital. J. Anim. Sci.*, **6**(Suppl.2): 965-967. DOI: 10.4081/ijas.2007.s2.965
- Sharma, D.K., P.K. Jallewar and K.K. Sharma. 2010. Antibiogram of bacteria isolated from bovine subclinical mastitis. *Indian Vet. J.*, **87**: 407.
- Sindhu, N., A. Sharma and V.K. Jain. 2010. Coagulase gene based molecular detection of *Staphylococcus aureus* directly from mastitic milk samples of Murrah buffalo. *Buffalo Bull.*, **29**(1): 52-59. Available on: <http://ibic.lib.ku.ac.th/e-Bulletin/IBBU201000009.pdf>
- Sudhakar, P., N. Awndkar and V. Khode. 2009. Prevalence and current Antibiogram trend of mastitic agents in Udgir and its vicinity, Maharashtra state, India. *International Journal of Dairy Science*, **4**(3): 117-122. DOI: 10.3923/ijds.2009.117.122
- Sumathi, B.R., B.M. Veeregowda and A.R. Gomes. 2008. Prevalence and antibiogram profile of bacterial Isolates from clinical bovine mastitis. *Vet. World*, **1**(8): 237-238. Available on: <https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.302.6846&rep=repl&type=pdf>
- Thapa, B.B. and K. Kaphle. 2002. Selecting different drug combinations for control of bovine clinical mastitis. *J. Anim. Vet. Adv.*, **1**: 8-11.
- Todhunter, D.A., L.L. Cantwell, K.L. Smith, K.H. Hoblet and J.S. Hogan. 1993. Characteristics of Coagulase negative *Staphylococci* isolated from bovine Intramammary infections. *Vet. Microbiol.*, **34**(4): 373-380. DOI: 10.1016/0378-1135(93)90062-C
- Tyagi, S.P. 2011. *Isolation and molecular characterization of Staphylococcus aureus mastitic dairy milk*. Thesis, Narendra Deva University of Agriculture and Technology, Uttar Pradesh, India.
- Tyagi, S.P., R.K. Joshi and N. Joshi. 2013. Characterization and antimicrobial sensitivity of *Staphylococcus aureus* isolates from subclinical bovine Mastitis. *Journal of Animal Health and Production*, **1**(2): 20-23. DOI: Available on: <http://www.nexusacademicpublishers.com/uploads/files/20130714005329.pdf>
- Wang, S., C. Wu, J. Shen, Y. Wu and Y. Wang. 2013. Hypermutable *Staphylococcus aureus* strains present at high frequency in subclinical bovine mastitis isolates are associated with the development of antibiotic resistance. *Vet. Microbiol.*, **165**(3-4): 410-415. DOI: 10.1016/j.vetmic.2013.04.009